Rearing Cotton Insects in the Laboratory

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Laboratory cultures of the following six Lepidoptera and two Coleoptera species are continuously maintained at the Western Cotton Insects Research Laboratory, Tucson, Ariz., as hosts for parasites and predators and as test insects for ecological, genetic, and nutritional studies: Bollworm (*Heliothis zea* (Boddie)), tobacco budworm (*Heliothis virescens* (F.)), beet armyworm (*Spodoptera exigua* (Hübner)), cabbage looper (*Trichoplusia ni* (Hübner)), salt-marsh caterpillar (*Estigmene acrea* (Drury)), pink bollworm (*Pectinophora gossypiella* (Saunders)), boll weevil (*Anthonomus grandis* Boheman), and thurberia weevil (*Anthonomus grandis thurberiae* Pierce). At certain times one Hemiptera species, *Lygus hesperus* Knight, is reared in the laboratory for similar purposes.

Five strains of a boll weevil complex and one strain of the thurberia weevil are reared in this laboratory. The boll weevil strains were originally collected from domestic cotton in various parts of Arizona and the thurberia weevil culture from wild cotton in the Santa Rita Mountains in this State.

Lepidoptera

The Lepidoptera species are maintained on a modified lima bean diet (*10*).[1] The main modifications are that baby lima beans are used instead of large lima beans and Gelcarin instead of agar.

The ingredients for a 1-gallon blender batch of diet are as follows:

Soaked baby lima beans	1,200 grams.
Brewer's yeast	120 grams.
Methyl-p-hydroxybenzoate	12 grams.
Ascorbic acid	12 grams.
Gelcarin	30 grams.
Water (total)	2,400 ml.
Formaldehyde	4 ml.

Six hundred grams of dry beans are soaked in 1,860 ml. of water for 18 to 20 hours. They can be put directly into the blender and are equivalent to 1,200 grams of soaked beans plus 1,200 ml. of water. Tap water from a well on the laboratory premises is used. The pH is about 7.2 and it is not known what contribution, if any, the salts in this water make.

Gelcarin is suspended by adding it to 1,200 ml. of hot water, blending it, and then heating to boiling. The boiling Gelcarin mixture is added to the soaked beans, brewer's yeast, methyl-p-hydroxybenzoate, formaldehyde, ascorbic acid, and water mixture, which has been blended for about 5 minutes to bring it to about 110° F. The higher temperature is necessary to keep the diet liquid so that the added hot Gelcarin will mix better and dispense readily. This method of mixing the heated diet has been used for about 3 years and was previously used with 50 grams of agar instead of the Gelcarin.

The current cost of ingredients per batch is about 90¢ with Gelcarin and about $1.45 with agar.

The hot diet is dispensed wih air pressure (*9*) into 9/16-, 1-, 8-, and 16-ounce cups (fig. 1). Diet for rearing the pink bollworm is poured directly from the blender into 1- by 17- by 27-inch sheet pans, with two pans per batch. It is allowed to dry at air temperature overnight and then is shredded by forcing it through 1/8-inch mesh hardware cloth (*8*). The shredded diet is allowed to air-dry an additional 24 hours before use.

Shorey and Hale (*11*) reported rearing nine species of noctuids on pinto bean diet. Although pinto beans are cheaper than the baby lima beans, the latter is used because all the species did not feed readily on the pinto bean diet. Larvae of the salt-marsh caterpillar do not develop "normally" on the pinto bean diet.

For all Lepidoptera species except the pink bollworm, moths are kept in gallon glass jars lined with a plastic bag, 3½ by 5 by 13 inches. The plastic bag is blown up inside the jar to fit the extremities of the jar. Pupae are sterilized and placed inside

[1] Italic numbers in parentheses refer to Literature Cited, p. 6.

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FIGURE 1.—Dispensing hot diet into 1-ounce cups with insect diet dispenser; 16-ounce cups on left.

the bag. The number varies with the species. Two strips of paper toweling about 1 inch wide are hung inside the bag and the top of the jar is covered with a 5-inch square of paper toweling, which is held in place with a cutout lid.

The jars are dated and the moths are allowed to emerge at 75° to 80° F. The jars are checked daily. When the first moth emerges, a watering vial, with the neck inserted through a hole in a 1-inch-square piece of Saran screen, is placed on top of the jar. This capless ½-dram screwcap vial is filled with 5-percent sugar solution. A V-shaped slit is cut in the toweling cover of the jar and the water vial is inverted on it. (Fig. 2.)

After the mating and preoviposition periods, which vary with each species, the jars are checked for eggs. When the first eggs are laid, the moths are transferred to clean plastic bags with clean toweling, and unemerged pupae are placed in clean jars or discarded. The moths are transferred to new bags daily and the watering vials are refilled each day and changed about every other day.

The toweling from the jars is collected daily after oviposition begins. The egg masses of the beet armyworm and the salt-marsh caterpillar are clipped from the paper for sterilizing to reduce the volume of paper to be washed and to speed drying. Areas of toweling containing no eggs of the bollworm, the tobacco budworm, and the cabbage looper are trimmed away to reduce the volume of paper to be washed. The eggs are surface-sterilized by the method described by Ignoffo (*7*).

The papers with the eggs are washed in a 0.3 percent sodium hyperchlorite solution in beakers or jars, depending on the number, and then they are rinsed in a 10 percent sodium thiosulfate solution and finally in water. Eggs that come loose from the papers can be recovered from the bottom of the container by swirling the contents. After they settle in the center, they can be removed with a pipette. They are air-dried, put in the same type plastic bag-lined jars to hatch as used for the moths, and covered with a cutout lid and tissue.

Pink bollworm pupae are sterilized, and about 600 are placed in each 1- by 1- by 1-foot screen cage. This number was chosen arbitrarily; fewer may be used. Pupae and moths are held at about 80° F. Cotton beauty sponges are used for watering and

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FIGURE 2.—Oviposition jar, showing plastic bag, toweling strips, and watering vial.

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FIGURE 3.—Pink bollworm emergence and oviposition cages, with cotton pads on top and cotton strips between cages.

for an oviposition surface. Two cotton sponges are placed on top of each cage. One is moistened with 5 percent sugar solution and the other with plain water. The moistening flattens the cotton to give it a better surface contact with the screen of the cage. The cage top and cotton sponges are then covered with wax paper.

The cages are placed on shelves, and an additional oviposition pad is provided between the cages. Absorbent cotton is cut into 1- by 12-inch strips. One of these strips is placed between two cages near the upper edge and the cages are brought together at the side to hold the cotton in place. This allows moths from adjoining cages to oviposit on the same strip. (Fig. 3.)

The cotton oviposition pads containing eggs of the pink bollworm are placed in a Büchner funnel and are soaked in a 1:200 formaldehyde solution for about 5 minutes. The excess solution is drawn off by vacuum and the pads are air-dried. The cotton strips from between the cages are washed in the same manner except that the outer layers bearing the eggs are separated from the center part to reduce the volume of cotton.

Bollworm

Larvae of the bollworm hatch in about 72 hours at 82° F. They are cannibalistic and must be reared individually. Two newly hatched larvae are placed with a camel's-hair brush in a 1-ounce plastic cup about two-thirds full of diet. The cup is closed with a center tab paper lid. A plastic lid can be used, but a high rate of mortality in the last larval instar results because of excessive moisture trapped in the cup. A higher rate of "cutting out" from the container by larvae occurs with this lid. The larvae pupate in about 14 days at 82°. Pupae are removed from the individual cups and then surface-sterilized in the same manner as described for the eggs (p. 2).

A continuous culture can be maintained by infesting seventy-five 1-ounce cups with larvae each day. Excess larvae, 25 to 30 per cup, are placed in 16-ounce cups closed with paper tab lids. These larvae are used as hosts for hymenopterous parasites when they reach the second or third instar. The yield is about 15 to 20 second- or third-instar larvae per cup.

About 20 pupae to provide adults for the successive generation are placed in the lined gallon jars described previously.

Tobacco Budworm

The larvae of the tobacco budworm also hatch in about 72 hours at 82° F. About 30 newly hatched larvae are placed with a camel's-hair brush on diet

in each 16-ounce cup, which is closed with a paper tab lid. Larvae are a suitable size for parasitization by tachinids in about 7 days and pupate in 12 to 14 days at 82°.

Pupae can be removed from their cells in the diet and frass by washing them from the cup with a sink spray hose into a basket of ⅛-inch mesh hardware cloth. The pupae do not float, but by gentle washing a large amount of frass can be removed so that the pupae can be easily removed from the basket by hand. Each cup will yield on an average about 12 pupae.

Beet Armyworm

The larvae of the beet armyworm hatch in about 48 hours at 82° F. Approximately 50 newly hatched larvae are placed with a camel's-hair brush in a 16-ounce cup filled about one-third with diet. The cup is closed with a paper tab lid. Larvae are a suitable size for parasitization after about 7 or 8 days at 82°.

The parent stock is maintained by retaining to pupation about 2 cups of larvae per day or every other day. Occasionally some slower developing larvae occur and their removal increases pupal yield because they feed on prepupae and freshly formed pupae.

The larvae pupate in 10 to 12 days and pupae are collected every other day. They are separated from the frass and diet by washing the contents of the rearing cup with a sink spray hose into a ⅛-inch mesh hardware cloth basket, 4 by 7 by 7 inches, placed in a 2- by 8- by 8-inch aluminum pan. The water accumulating in the pan allows the pupae to float to the surface and the frass and diet to sink to the bottom. The pupae are then skimmed off the surface with a screen sieve. Pupae that sink can be brought to the surface by lifting the basket out of the water and then dipping it into the water. The pupae are surface-sterilized in the same manner as described for the eggs (p. 2).

About 25 pupae are placed in plastic bags inside gallon emergence jars as previously described. Moths emerge in about 6 days at 78° F. and begin to lay eggs after 1 or 2 days. Sexing the pupae is unnecessary because the sex ratio is about even. Moths lay eggs in appreciable numbers for about 4 or 5 days and are then discarded.

Cabbage Looper

The larvae of the cabbage looper hatch in about 72 hours at 82° F. About 35 newly hatched larvae are placed with a camel's-hair brush on diet in a 16-ounce cup closed with a paper tab lid. Larvae pupate in about 12 to 14 days around the top and sides of the cup and under the lid.

About 25 pupae are obtained from each cup. These are handpicked from the cocoons, although they may be washed with sodium hypochlorite solution to dissolve the cocoons. Pupae are then surface-sterilized in the same manner as described for the eggs (p. 2). The pupae are collected 3 days a week, and about 20 are placed in emergence jars to provide adults for the next generation.

Salt-Marsh Caterpillar

The larvae of the salt-marsh caterpillar hatch in about 96 hours at 82° F. About 25 larvae are placed with a camel's-hair brush on diet in a 16-ounce cup covered with a paper tab lid. The cups are placed on their side so that frass from the feeding larvae drops away from the diet. About 15 to 20 larvae suitable for parasitization by tachinids are obtained after 14 days at 82°. At the same time 14-day-old larvae are placed in thirty 1-ounce cups of diet every other day, with one larva per cup for parent stock. These larvae pupate in about 6 more days. Although they are generally not cannibalistic, some cannibalism occurs if they are left in 16-ounce cups to pupate and vary in size, with younger larvae eating freshly formed pupae.

Pupae are collected from the 1-ounce cups and surface-sterilized by the same procedure as described for the eggs (p. 2). About 15 to 20 are placed in each gallon emergence jar and adults emerge 8 to 10 days later at 78° F.

Pink Bollworm

Larvae of the pink bollworm hatch in about 96 hours at 82° F. They are reared either individually or in numbers. For those reared individually, two or more newly hatched larvae are placed with a camel's-hair brush in a 9/16-ounce cup about half full of diet and capped with a plastic lid. Although two newly hatched larvae are placed in each cup, more than one pupa is rarely recovered because of cannibalism. The amount of diet is more than larvae will eat, but less diet is unsatisfactory because it dries out. Rearing is better when cups are placed upside down after capping. This serves a double purpose: First, newly hatched larvae climb upward and come into contact with the diet; second, the larvae pupate on the lid instead of burrowing into the diet and thus removal is easier.

Numbers of larvae are reared in 1-gallon ice cream cartons with the shredded diet described previously (p. 1). Hatching larvae or eggs about to hatch on the cotton pads are placed between alternate ⅜-inch layers of diet and cotton. The cotton serves to trap larvae between layers of diet and later to isolate them from each other.

After about 10 days at 86° F., larvae begin to

cut out of the cartons. At this time the diet is separated from the cotton and placed in Berlese funnels. No heat is applied. A folded facial tissue is placed in the jars attached to the funnels. The larvae drop down into these collection jars and form pupal cells between the layers of facial tissues. Pupae are removed by separating the layers of tissue, which opens the cells. Pupae are surface-sterilized with a 0.3 percent sodium hypochlorite solution.

Coleoptera

Boll Weevil and Thurberia Weevil

The adults of the boll weevil and the thurberia weevil are fed on paraffin-waxed pellets made by the method and with the diet described by Gast (*5*, *6*). They feed on and oviposit in the pellets. They are kept in plastic shoe boxes, 3½ by 6¾ by 11½ inches, with about 300 per box. The bottom of the box is lined with paper towels to keep it cleaner and to give the weevils a place to hide when not feeding. The pellets are changed every other day.

The first step in recovering eggs from the pellets is to remove the paraffin shells from the pellets by adding water and agitating them in a household-type electric mixer on low and medium speeds for about 5 minutes. The wax breaks off and floats to the top of the water. The diet with the eggs sinks to the bottom. The wax is then skimmed off with a fine mesh screen.

The diet is washed through a series of 14-, 20-, 30-, and 40-mesh sieve screens, as described by Gast (4). The eggs and fine diet particles are left in the bottom 40-mesh screen. They are collected from the screen and the excess water is drained off. The eggs are then separated from the diet by flotation in a saturated salt solution (*4*). The eggs and diet particles are poured into a separatory funnel and allowed to stand for about 5 minutes. The diet sinks to the bottom and the eggs float to the top of the solution. The diet is drawn out at the bottom of the funnel and only the eggs are left in the solution.

The eggs are drawn off into a beaker and washed in a 1:50 formaldehyde solution for sterilization. When the formaldehyde solution is added, the eggs sink to the bottom of the container. They are removed by swirling the solution, allowing them to come to rest in the center of the beaker, and then drawing them off with a pipette. They are placed in a 1:200 solution of formaldehyde. From this solution they are implanted on the diet with a disposable capillary pipette by the method described by Betz (*1*).

The larvae are reared on the cottonseed meal diet reported by Sterling and Adkisson (*12*). The heated diet is poured into 15- by 100-mm. disposable plastic petri dishes with a pressure paint tank diet dispenser described by Patana (*9*). The dishes are covered and stored at room temperature. Before the eggs are implanted in the dishes, the diet surface is scratched with a triple-bladed knife similar to that described by Betz (*1*) to give the larvae a feeding surface. Instead of implanting the eggs directly on the surface of the diet, the eggs are dropped and scattered on a single thickness of facial tissue placed inside the lid of the dish and excess water is drained off. The lid is placed over the dish. When the eggs hatch, the larvae drop to the diet surface and begin feeding.

The development from egg to adult varies with the temperature, averaging about 33.1 days at 68° F., about 21 days at 77°, and 16.9 days at 86°. Since the laboratory rearing is done at about 80°, between 17 and 20 days are required for development.

Hemiptera

Lygus Bug

Laboratory cultures of a lygus bug, *Lygus hesperus* Knight, have been reared by the method described by Bottger (*2*). Fresh green beans are used for food and for an oviposition medium.

Parent cultures can be collected from alfalfa or from certain weeds with a sweep net. Adults can be separated from trash and other insects in a flight box consisting of a darkened box with one side covered with nylon or translucent plastic and an open side covered with a dark drop cloth. As the lygus bugs are attracted to the lighter side, they can be aspirated by a vaccum connected to the vehicle manifold.

Oviposition cages are 12- by 8- by 8-inch wood frames covered with 30-mesh Saran screen on three sides and a sliding glass door on the fourth. The top and bottom are solid. One hundred to two hundred lygus bugs of each sex are placed in the cage. Three or four fresh green beans are placed on the bottom of the cage. One-gallon jars can also be used for oviposition cages. The females are allowed to oviposit on the beans for about 2 days and the beans are then changed and replaced with fresh ones. When removing the beans from the oviposi-

tion cage, if the cage or jar is placed in a window, the adults can be shaken from the beans and they will fly toward the light resulting in fewer escaping. This can also be accomplished in a dark room by placing a light behind the cage or jar.

The beans in which the adults have oviposited are transferred to a 1-gallon jar, which is dated and covered with a 6-inch square of muslin held in place with a rubberband. The eggs hatch in about 6 days at 78° F. Development times for eggs and nymphs at constant and fluctuating temperatures were reported by Champlain and Butler (*3*). At 4, 6, and 8 days about two fresh beans are added to the jar to provide food for the newly hatched nymphs. At 10 days the old beans are removed and fresh beans provided for the nymphs every other day thereafter. When the beans are changed, the nymphs can be dislodged from them by gently tapping the beans against the side of the jar. The nymphal period requires about 16 to 18 days at 78° and a relative humidity of 50 to 60 percent. The preoviposition period is about 6 days and egg laying about 2 weeks.

Mold on the beans, which may at times become severe, can be partially avoided by selecting only fresh undamaged beans for oviposition and feeding. Another problem sometimes is insecticide contamination of beans. One method of overcoming both difficulties is to wash the beans.

A cylindrical ½-inch mesh hardware cloth basket about 1 foot in diameter is used. The washing solutions can be made up in 5-gallon pails. The beans in the basket are immersed in detergent solution, agitated, lifted out, and the excess solution is drained off. This will remove the insecticide and probably some of the mold spores. The beans are dipped into a 1 percent Phaltan solution and allowed to drain. They are then spread out and air-dried using a fan to speed up drying. When dry, they can be placed in the refrigerator until needed.

Summary

Larvae of the bollworm (*Heliothis zea* (Boddie)), the tobacco budworm (*Heliothis virescens* (F.)), the beet armyworm (*Spodoptera exigua* (Hübner)), the cabbage looper (*Trichoplusia ni* (Hübner)), the salt-marsh caterpillar (*Estigmene acrea* (Drury)), and the pink bollworm (*Pectinophora gossypiella* (Saunders)) are reared on the same diet in the laboratory. However, rearing procedures vary, as some of these lepidopterous species are cannibalistic and have slightly different feeding and pupating characteristics. Techniques for handling moths of the various species for oviposition are similar except for the pink bollworm.

Larvae of the boll weevil complex (*Anthonomus grandis* Boheman) and the thurberia weevil (*Anthonomus grandis thurberiae* Pierce) are reared on the same diet, although it differs from that of the lepidoperous larvae. In addition, adults of these two coleopterous species have to be fed a diet, whereas a sugar solution suffices for the moths.

A lygus bug, *Lygus hesperus* Knight, can be reared in the laboratory using fresh green beans for food and for an oviposition medium.

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